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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/505,252	08/19/2004	Francois Romagne	INN-112	7415

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EXAMINER	
FORD, ALLISON M	

ART UNIT	PAPER NUMBER
1651	

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Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Office Action Summary	Application No.	Applicant(s)	
	10/505,252	ROMAGNE ET AL.	
	Examiner	Art Unit	
	ALLISON M. FORD	1651	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *09 July 2007 and 14 September 2007*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *21,23-25 and 27-52* is/are pending in the application.

4a) Of the above claim(s) *35-50* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *21,23-25 and 27-34* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☒ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☒ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☒ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date *20070709*.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____.
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

DETAILED ACTION

Request for Continued Examination

A request for continued examination under 37 CFR 1.114, including the fee set forth in 37 CFR 1.17(e), was filed in this application after final rejection. Since this application is eligible for continued examination under 37 CFR 1.114, and the fee set forth in 37 CFR 1.17(e) has been timely paid, the finality of the previous Office action has been withdrawn pursuant to 37 CFR 1.114. Applicant's submissions filed on 7/9/2007 and 9/14/2007 have been entered.

Priority

Acknowledgment is made of the instant application being a 371 application of international application PCT/FR03/000585 on 2/21/2003. Acknowledgement is furhter made of the claim for foreign priority under 35 USC 119(a)-(d) to application FR 02/02305, filed 2/22/2002; a certified copy of the foreign priority documents have been received in the instant application.

Response to Arguments

Applicants' arguments received with the response of 9/14/2007 have been fully considered, but are not found persuasive to overcome the rejection of record.

Regarding the rejection of claims 21-34 under 35 USC 103(a), as being unpatentable over Belmant et al or Espinosa et al, each in view of Garcia et al and Valeri, Applicants have reiterated their arguments that the claimed method, which requires a cell density of less than about 5×10^6 cells/mL be maintained during the culture step, produces unexpected results as far as percentage gamma delta T cells obtained in the cell culture. Applicants assert these unexpected and superior results are indicative of non-

obviousness. Applicants further assert that the duration of the cell culture period would not ensure a higher proportion of gamma delta T lymphocytes.

In response to Applicants' argument that the claimed culture concentration yields unexpected results, it is respectfully submitted that newly cited Skea et al has been incorporated into the rejection of record to support that the culture concentrations currently claimed were routine values for culture of gamma delta T lymphocytes (see rejection below). Thus the culture concentration levels currently claimed are not unique in view of the cited art, and any particular benefit of the claimed concentration (such as proportion of gamma delta T lymphocytes obtained), though not expressly recognized by the prior art, are still rendered obvious. See *In re Dillon*, 919 F.2d 688, 16 USPQ2d 1897 (Fed. Cir. 1991).

Furthermore, it is noted a showing of unexpected results must be based on evidence, not argument or speculation. *In re Mayne*, 104 F.3d 1339, 1343-44, 41 USPQ2d 1451, 1455-56 (Fed. Cir. 1997). It appears Applicants are relying on Examples IIA & Table 8 of the instant specification; however, these specific examples do not show that the concentration of gamma delta T lymphocytes varies with changing cell densities. Rather the results show that cultures maintained at three different cell densities: 2×10^5 cells/mL, 5×10^5 cells/mL, 1.5×10^6cells/mL, all achieved a gamma delta T lymphocyte population of 94%; these results, while sufficient to show operability of the method, does not provide evidence that the same cell cultures, when maintained at cell densities greater than 5×10^6 cell/mL were incapable of achieving such gamma delta T lymphocyte populations. Though applicants point to the results of Belmant et al and Espinosa et al, they fail to particularly point out which specific results or experiments they are relying upon to show inconsistencies. Neither Belmant et al nor Espinosa et al were asserted to anticipate the claimed invention, rather, the rejection is based on the obviousness of the instant invention, including the culture parameters (e.g. cell density), over the cited references. It is further noted

that, with the exception of claim 34, none of the claims require the method produce a cell population that is at least 80% gamma delta T lymphocytes.

In response to Applicants' argument that the duration of cell culture does not necessarily affect the density or percentage of gamma delta T cells in the cell culture, the rejection of record has been modified to incorporate the teachings of Skea et al, who expressly show that the duration of culture, up to at least 27 days, does have an increasing linear relationship with the number of gamma delta T cells in the cell culture (See rejection below).

Claim Rejections - 35 USC § 112

The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Claims 21, 23-25, 27-34, 51 and 52 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

In claim 21, it is not clear if the 'biological preparation' initially contains gamma delta T lymphocytes, and the method is intended to enrich the proportion of gamma delta T lymphocytes within the biological preparation, or if the biological preparation may contain any mononuclear cell population, and the method is intended to involve transdifferentiation of various (non-gamma delta T lymphocytes) mononuclear cells into gamma delta T lymphocytes. As is, the claims do not currently make the intent of the method clear, or the steps necessary to achieve the method, if a transdifferentiation step is required such must be expressly claimed. Clarification is required.

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

Claims 21, 23-25, 27-34 and 51-52 are rejected under 35 U.S.C. 103(a) as being unpatentable over Belmant et al (US Patent 6,660,723), in view of Skea et al (Journal of Hematotherapy and Stem Cell Research, Oct 2001), Garcia et al (Journal of Immunology, 1998) and Valeri (Blood Banking and the Use of Frozen Blood Products, 1976).

Belmant et al teach a method for enriching the concentration of gamma delta T lymphocytes (Tγ9δ2 lymphocytes) in a cell sample (which Applicants call preparing a gamma delta T lymphocyte composition), comprising providing a biological preparation comprising gamma delta T lymphocytes, and culturing the biological preparation with a phosphohalohydrin and interleukin-2 (IL-2) (See Belmant et al, col. 2, ln 44-65 & specifically Example 10, col. 22, ln 1-34). The phosphohalohydrin 3-(bromomethyl)-3-butanol-1-yl-diphosphate (BrHPP) is exemplified in Example 10. The biological preparation provided in Example 10 is a blood extract, which is considered to read on a blood sample. (relevant to claims 21, 29-32, 51)

Belmant et al differ from the instant invention in that they do not disclose the same specific culture parameters recited in the instant claims, including the original cell count, cell density during culture, culture duration, or concentration of the cytokine. However, it would have been well within the purview of one of ordinary skill in the art to optimize the duration of the culture period, the cell density before and during culture, and the concentration of the activator compounds (BrHPP and IL-2) to those values recited in the instant claims, as each of the parameters was recognized in the art as acceptable

values for T lymphocyte cell culture, and/or were recognized as result effective variables, which would have been routinely optimized by the artisan of ordinary skill.

With regards to the cell count of the initial cell sample, it is submitted that, in the field of cell culture, the starting cell count is generally recognized to be a result effective variables that directly affect the final cell number produced by the culture. A sufficient number of cells must be initially provided to establish a primary culture. The number of cells provided is less important than the concentration of cells provided; thus, depending on the scale of the culture to be carried out, the cell count of the initial biological sample would have been routinely optimized to provide the desired concentration (cells/mL). In Example 10, Belmant et al disclose providing 10^6 T lymphocytes in a 1 mL sample (at least 1 million mononuclear cells/mL) (See Belmant et al, col. 22, ln 1-10). However, in order to scale up the culture, 50-100 mL of biological sample may be provided, which at the same concentration, would provide 50-100 mononuclear cells, as currently required by claims 21 and 24.

With regards to the concentration maintained throughout the culture period, Belmant et al is silent as the culture concentration; however it is noted that Skea et al, also directed to culture of T lymphocytes, report providing cells at an initial concentration of 1×10^5 cells/mL, and passaging cells every 4-7 days to the same concentration (See Skea et al, Pg. 526, col. 1, "T Cell Culture"). Thus, while Belmant et al is silent as to the cell culture density, there was teachings in the art at the time the invention was made that show T cell cultures, including gamma delta T cells, are successfully cultured at a concentration of 1×10^5 cells/mL, which is within the range currently required by claim 21. As such, one of ordinary skill in the art would have been motivated to follow the teachings of Skea et al and passage the culture of Belmant et al, as necessary to maintain the disclosed cell density.

With regards to the duration of culture, while Belmant et al report culturing the cells for 8 days, it is submitted that the duration of the culture was recognized as directly affecting the final percentage of gamma delta-positive cells in the final culture, see Skea et al. Skea et al, directed to the culture of T

lymphocytes, including gamma delta T lymphocytes for enrichment of gamma delta-positive cells, shows that the percentage of gamma delta-positive cells increased linearly over time, up to 27 days (See Skea et al, Pg. 534, Fig. 7); thus supporting the assertion that duration of culture is a result effective variable. Therefore, because the duration of culture directly affects the degree of enrichment of the gamma delta T cells, and the method of Belmant et al is intended to produce an enriched population of delta gamma T cells, one of ordinary skill in the art would have found it *prima facie* obvious to extend the culture period of Belmant et al including the time periods recited in claims 27 and 28, as a matter of routine optimization, to improve the target cell yield.

Finally, with regards to the concentration of the IL-2 provided in the culture, while Belmant et al disclose using 50 U/mL of IL-2, it is submitted that the concentration of the cytokines was recognized as a result effective variable that would have been routinely optimized by the artisan of ordinary skill. Belmant et al specify the concentration of the IL-2 is to be "in a proportion suitable to bring about lymphocyte growth" (See Belmant et al, col. 12, ln 5-7); therefore, while applicant specifically claims a cytokine concentration in the range of 150 U/mL to 500 U/mL, manipulation and optimization within this range would be well within the purview of one of ordinary skill in the art, again based on the desired activation rate of the cells as well as how often the cells are passaged (Claim 33). Generally, differences in concentration will not support the patentability of subject matter encompassed by the prior art unless there is evidence indicating such concentration is critical or produces unexpected results. Where the general conditions of a claim are disclosed by the prior art it is not inventive to discover the optimum or workable ranges by routine experimentation, See *In re Aller,* 220 F.2d 454, 456, 105 USPQ 233, 235 (CCPA 1955).

Still further, regarding the cytokines used in the experiments of Belmant et al, while Belmant et al added IL-2 to their media, it would further have been obvious to one of ordinary skill in the art at the time the invention was made to alternatively add IL-15 to the culture medium, as recited in claims 21 and 52. In support see Garcia et al; Garcia et al teach that specific cytokines, including IL-2 and IL-15 are known to enhance gamma delta T lymphocyte activation in the presence of an activator of gamma delta T cells (See Garcia et al, Pg. 4324 & Fig. 2). Garcia et al unexpectedly found IL-15 to be a more potent induces of gamma delta T cell proliferation than IL-2 (See Garcia et al, Pg. 4324, col. 2). Therefore, it would have been well within the purview of one of ordinary skill in the art to alternatively utilize IL-15 in place of, or in addition to, the IL-2 used by Belmant et al (Claims 21 & 32). One of ordinary skill in the art would be motivated to substitute IL-15 for IL-2 in the method of Belmant et al because Garcia et al teach IL-15 is more potent than IL-2 in enhancing gamma delta T cell proliferation. One would expect success based on the experimental findings of Garcia et al, which show successful activation and proliferation of gamma delta T cells in the presence of IL-15 and an activator compound. Regarding the concentration of IL-15 used, Garcia et al use of up to 100 ng/mL of IL-15, but report the effect of IL-15 on gamma delta T cell proliferation was dose dependent (See Garcia et al, Pg. 4324, col. 2). Therefore, while applicant specifically claims a cytokine concentration in the range of 150 U/mL to 500 U/mL, manipulation and optimization within this range would be well within the purview of one of ordinary skill in the art, again based on the desired activation rate of the cells as well as how often the cells are passaged (Claim 33).

Finally, while Belmant et al teaches the gamma delta T lymphocytes can be from a blood sample or blood extract, they do not specifically teach separating whole blood by cytapheresis prior to the culture method described above, nor do they teach using previous frozen samples of blood. However, at the time the invention was made it would have been well within the purview of one of ordinary skill in the art to obtain a blood sample at a first point in time, separate the whole blood sample by cytapheresis into

individual components, freeze the individual components by appropriate means known in the art, and then at later time, thaw the desired platelet component for use in the method of Belmant et al (Claims 23 and 25). In support see Valeri; Valeri teaches the basic protocols for obtaining, separating and storing blood. Valeri teaches cytapheresis allows separation of specific components of blood from whole blood (See Valeri, Pg. 1, col. 1). Separating the platelets via 'plateletpheresis' removes the majority of red blood cells and plasma components, thereby increasing the proportion of gamma delta T lymphocytes present in the initial cell sample, which is desirable in the method of Belmant et al, as it would ensure a higher proportion of gamma delta T lymphocytes for culture and expansion, resulting in a greater end cell count. Additionally, Valeri teaches methods for frozen storage of separated platelets (See Valeri, Pg 297), which is desirable when the blood sample must be stored for a period of greater than 4 hours prior to use in the method of Belmant et al (See Valeri, Pg. 6, col. 1). One would expect success separating the desired platelet component via cytapheresis and storing the separated components in a frozen state for future use, because such methods and procedures are common in the art of hematology, as demonstrated by the teachings of Valeri.

Therefore the invention as a whole would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention was made.

Claims 21, 23-25, 27-34 and 51-52 are rejected under 35 U.S.C. 103(a) as being unpatentable over Espinosa et al (Journal of Biological Chemistry, 2001), in view of Skea et al (J Hematotherapy & Stem Cell Res, 2001), Garcia et al (Journal of Immunology, 1998) and Valeri (Blood Banking and the Use of Frozen Blood Products, 1976).

Espinosa et al sought to identify a synthetic activator of gamma delta T lymphocytes that has comparable immunostimulatory activity as natural phosphoantigens; Espinosa et al discovered BrHPP enabled immunostimulation of human gamma delta T lymphocytes (See Espinosa et al, abstract).

Espinosa et al first perform a control run using the known, natural phosphoantigen 3-formyl-1-butyl-pyrophosphate (3fbPP); peripheral blood lymphocytes were cultured at an initial concentration of 10^6 cells/mL in the presence of 10nM 3fbPP and 100 U/mL IL-2 for a 15 day period (See Espinosa et al, Pg. 18338, col. 1). Espinosa et al report significant expansion of the gamma delta T lymphocytes, including compositions comprising greater than 95% TCR Vδ2 positive cells (gamma delta T lymphocytes) (See Espinosa et al, Pg. 18338, col. 2).

Espinosa et al then perform an experimental run using several different concentrations (12.5, 25, 100 nM) of BrHPP as the activator instead of the natural 3fbPP (See Espinosa et al, Pg. 18340, col. 1-2 & Fig. 4).

Espinosa et al do not specifically describe the culture conditions of the experimental run, while they do state that peripheral blood cells were used, they are silent on the initial cell count, the length of the culture period, and whether or not IL-2 was added to the culture. However, it appears the culture conditions for the experimental run were identical to the conditions of the control run: 10^6 cells/mL were present in initial culture as well as 100 U/mL of IL-2, and the culture was maintained for 15 days. One of ordinary skill in the art would assume that for results to be comparable between the immunostimulatory activity of the 3fbPP and BrHPP, the culture conditions were identical. Therefore, in the absence of evidence to the contrary, it is assumed Espinosa et al performed a method for activation of a gamma delta T lymphocyte composition comprising culturing peripheral blood lymphocytes (PBL) in the presence of BrHPP (a synthetic activator of gamma delta T lymphocytes) and IL-2.

However, even if the culture conditions described for the control run were not duplicated in the experimental run, it would have been well within the purview of one of ordinary skill in the art to optimize the duration of the culture period, the cell density before and during culture, and the concentration of the activator compounds (BrHPP and IL-2) to those values recited in the instant claims, as each of the parameters was recognized in the art as acceptable values for T lymphocyte cell culture, or

were recognized as result effective variables, which would have been routinely optimized by the artisan of ordinary skill.

With regards to the cell count of the initial cell sample, it is submitted that, in the field of cell culture, the starting cell count is generally recognized to be a result effective variables that directly affect the final cell number produced by the culture. A sufficient number of cells must be initially provided to establish a primary culture. The number of cells provided is less important than the concentration of cells provided; thus, depending on the scale of the culture to be carried out, the cell count of the initial biological sample would have been routinely optimized to provide the desired concentration (cells/mL).

Espinosa et al disclose providing an initial cell culture of 10^6 cells/mL; however, in order to scale up the culture, greater amounts of biological sample may be provided, which at the same concentration, would provide 50-100 mononuclear cells, as currently required by claims 21 and 24.

With regards to the concentration maintained throughout the culture period, Espinosa et al is silent as the concentration throughout culture; however it is noted that Skea et al, also directed to culture of T lymphocytes, report providing cells at an initial concentration of 1×10^5 cells/mL, and passaging cells every 4-7 days to the same concentration (See Skea et al, Pg. 526, col. 1, "T Cell Culture"). Thus, while Espinosa et al is silent as to the cell culture density, there was teachings in the art at the time the invention was made that show T cell cultures, including gamma delta T cells, are successfully cultured at a concentration of 1×10^5 cells/mL, which is within the range currently required by claim 21. As such, one of ordinary skill in the art would have been motivated to follow the teachings of Skea et al and passage the culture of Espinosa et al, as necessary to maintain the disclosed cell density.

With regards to the duration of culture, while Espinosa et al report culturing the 3fbPP for 15 days, they do not recite the culture duration with BrHPP; however, it is submitted that the duration of the culture was recognized as directly affecting the final percentage of gamma delta-positive cells in the final culture, see Skea et al. Skea et al, directed to the culture of T lymphocytes, including gamma delta T

lymphocytes for enrichment of gamma delta-positive cells, shows that the percentage of gamma delta-positive cells increased linearly over time, up to 27 days (See Skea et al, Pg. 534, Fig. 7); thus supporting the assertion that duration of culture is a result effective variable. Therefore, because the duration of culture directly affects the degree of enrichment of the gamma delta T cells, and the method of Espinosa et al is intended to produce an enriched population of delta gamma T cells, one of ordinary skill in the art would have found it *prima facie* obvious to extend the culture period of Espinosa et al including the time periods recited in claims 27 and 28, as a matter of routine optimization, to improve the target cell yield.

Finally, the concentration of the activator compounds, both BrHPP and IL-2, would also directly effect the rate of proliferation and expansion of the gamma delta T lymphocytes in the culture. For example, Espinosa et al teach the concentration of the IL-2 to be 100 U/mL (See Espinosa et al, Pg. 18338, col. 1); however, while applicant specifically claims a cytokine concentration in the range of 150 U/mL to 500 U/mL, manipulation and optimization within this range would be well within the purview of one of ordinary skill in the art, again based on the desired activation rate of the cells as well as how often the cells are passaged (Claim 33). Generally, differences in concentration will not support the patentability of subject matter encompassed by the prior art unless there is evidence indicating such concentration is critical or produces unexpected results. Where the general conditions of a claim are disclosed by the prior art it is not inventive to discover the optimum or workable ranges by routine experimentation, See *In re Aller*, 220 F.2d 454, 456, 105 USPQ 233, 235 (CCPA 1955).

It then naturally follows that because all of the above discussed variables directly effect the final gamma delta T lymphocyte cell count and concentration in the final composition, it would have been within the purview of one of ordinary skill in the art to optimize any and each of the variables so as to produce a composition with any desired cell concentration and cell count, including at least 80% gamma delta T lymphocytes and at least 100 million cells. In the instant reference Espinosa et al report a final gamma delta T lymphocyte population which comprises approximately 63% of total lymphocytes (See

Espinosa et al, Fig. 4a); alternatively, Espinosa et al teach the concentration of BrHPP directly effects the final gamma delta T lymphocyte count (See Pg. 18340, col. 2 & Fig. 4B). Thus one would have expected success optimizing the cell count and gamma delta T lymphocyte cell proportion because these variables are directly controlled by the culture steps and parameters discussed above, particularly the concentration of BrHPP; it would be well within the purview of one of ordinary skill in the art to optimize the culture conditions (extend culture time period, adjust time between passages, adjust concentration of activation agents) in order to create a gamma delta T lymphocyte composition with the desired cell numbers and proportions (Claim 34).

Still further, regarding the cytokines used in the experiments of Espinosa et al, while Espinosa et al added IL-2 to their media, it would further have been obvious to one of ordinary skill in the art at the time the invention was made to alternatively add IL-15 to the culture medium. In support see Garcia et al; Garcia et al teach that specific cytokines, including IL-2 and IL-15 are known to enhance gamma delta T lymphocyte activation in the presence of an activator of gamma delta T cells (See Garcia et al, Pg. 4324 & Fig. 2). Garcia et al unexpectedly found IL-15 to be a more potent induces of gamma delta T cell proliferation than IL-2 (See Garcia et al, Pg. 4324, col. 2). Therefore, it would have been well within the purview of one of ordinary skill in the art to alternatively utilize IL-15 in place of, or in addition to, the IL-2 used by Espinosa et al (Claims 21 & 32). One of ordinary skill in the art would be motivated to substitute IL-15 for IL-2 in the method of Espinosa et al because Garcia et al teach IL-15 is more potent than IL-2 in enhancing gamma delta T cell proliferation. One would expect success based on the experimental findings of Garcia et al, which show successful activation and proliferation of gamma delta T cells in the presence of IL-15 and an activator compound. Regarding the concentration of IL-15 used, Garcia et al use of up to 100 ng/mL of IL-15, but report the effect of IL-15 on gamma delta T cell proliferation was dose dependent (See Garcia et al, Pg. 4324, col. 2). Therefore, as discussed above, while applicant specifically claims a cytokine concentration in the range of 150 U/mL to 500 U/mL,

manipulation and optimization within this range would be well within the purview of one of ordinary skill in the art based on the desired activation and proliferation rate of the cells as well as how often the cells are passaged (Claim 33).

Finally, while Espinosa et al teach use of peripheral blood lymphocytes, they do not specifically teach separating whole blood by cytapheresis prior to the culture method described above, nor do they teach using previous frozen samples of blood. However, at the time the invention was made it would have been well within the purview of one of ordinary skill in the art to obtain a blood sample at a first point in time, separate the whole blood sample by cytapheresis into individual components, freeze the individual components by appropriate means known in the art, and then at later time, thaw the desired platelet component for use in the method of Espinosa et al (Claims 23 and 25). In support see Valeri; Valeri teaches the basic protocols for obtaining, separating and storing blood. Valeri teaches cytapheresis allows separation of specific components of blood from whole blood (See Valeri, Pg. 1, col. 1). Separating the platelets via 'plateletpheresis' removes the majority of red blood cells and plasma components, thereby increasing the proportion of gamma delta T lymphocytes present in the initial cell sample, which is desirable in the method of Espinosa et al, as it would ensure a higher proportion of gamma delta T lymphocytes for culture and expansion, resulting in a greater end cell count. Additionally, Valeri teaches methods for frozen storage of separated platelets (See Valeri, Pg 297), which is desirable when the blood sample must be stored for a period of greater than 4 hours prior to use in the method of Espinosa et al (See Valeri, Pg. 6, col. 1). One would expect success separating the desired platelet component via cytapheresis and storing the separated components in a frozen state for future use, because such methods and procedures are common in the art of hematology, as demonstrated by the teachings of Valeri.

Therefore the invention as a whole would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention was made.

Conclusion

Any inquiry concerning this communication or earlier communications from the examiner should be directed to ALLISON M. FORD whose telephone number is (571)272-2936. The examiner can normally be reached on 8:00-6 M-Th.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Michael Wityshyn can be reached on 571-272-0926. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/Allison M. Ford/
Examiner, Art Unit 1651